THE EFFECT OF DIMETHYL SULFOXIDE ON ENDOTOXIN-INDUCED PULMONARY DYSFUNCTION: A BIOCHEMICAL AND ELECTRON MICROSCOPE STUDY

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INTRODUCTION

The administration of endotoxin in experimental animals has been shown to cause alterations in pulmonary capillary endothelial cell (PCEC) metabolic activity and ultrastructure.[1,2,4,7] These changes occur in the presence of intravascular accumulations of platelets and leukocytes. It has been postulated that lysosomal enzymes and/or highly reactive O_2 metabolites released from blood elements may be responsible for these cytotoxic changes.[5,8] The present study was designed to evaluate dimethyl sulfoxide (DMSO), a known scavenger of free radicals, with regard to its protective effect on PCEC function and fine structure and on survival in endotoxin-treated rats.

MATERIALS AND METHODS

Fifty-four male Sprague Dawley rats weighing between 300 and 500 g were divided into three groups. The first group of 10 animals served as normal controls and were assayed for PCEC luminal adenosine triphosphatase (ATPase) activity. The second and third groups consisting of 22 animals each were given an intraperitoneal injection of 6 ml/kg of either saline or 70% DMSO 30 minutes prior to an intravenous injection of 20 mg/kg of *E. coli* endotoxin (Sigma-serotype 026:B6). Ten rats each from groups 2 and 3 were assayed for ATPase activity at 4 h and the survivors of the remaining 12 rats in each of the groups were assayed at 24 h. Lung biopsies were taken at 24 h from additional animals in groups 2 and 3 and prepared for an electron microscopic examination. At the time of assay the isolated heart and lungs were flushed with normal saline to remove the blood. Once the effluent was clear, the lungs were perfused with a modified Wachstein-Miesel solution for the demonstration of luminal ATPase activity.[9] The first 5-minute collection of effluent was discarded and the subsequent 5-minute collection was assayed for inorganic phosphorus (P_i) using the method of Fiske and Subbarow.[3] The lungs were removed and dry weights determined. The amount of P_i liberated was used as a marker for PCEC metabolic activity and expressed as mg P_i per ml effluent per gram of dry lung tissue (mg/ml/g).

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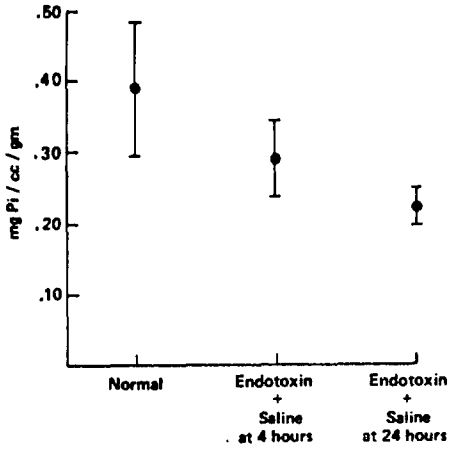

FIGURE 1. The mean P_i level was .390 ± .095 for normal rats. The amount of P_i assayed at 4 and 24 h was .288 ± .052 and .221 ± .024 for the rats given an intravenous injection of endotoxin (20 mg/kg) and an intraperitoneal injection of 6 ml/kg of saline.

RESULTS

The mean amount of P_i liberated after perfusion of isolated heart and lungs with the ATP-containing buffer in normal rats was .390 ± .095. In the saline/endotoxin-treated group the P_i levels were decreased at both 4 and 24 h (.288 ± .052 and .221 ± .024) compared to normal rats ($p < .005$). In the DMSO/endotoxin-treated group the P_i levels at 4 h were not significantly different from normal rats (.367 ± .057) but at 24 h were somewhat lower than normal (.315 ± .113, $p < .1$, FIGURES 1 and 2). At 24 h, the lungs of the saline/endotoxin-treated rats exhibited microvascular aggregations of leukocytes and degeneration of the capillary endothelium and overlying alveolar type I epithelium. These ultrastructural lesions were not observed in rats treated with DMSO. All 12 DMSO/endotoxin-treated rats survived 24 h (100%) compared to only 7 of the 12 saline/endotoxin-treated rats (58%, $p < .025$, FIGURE 3).

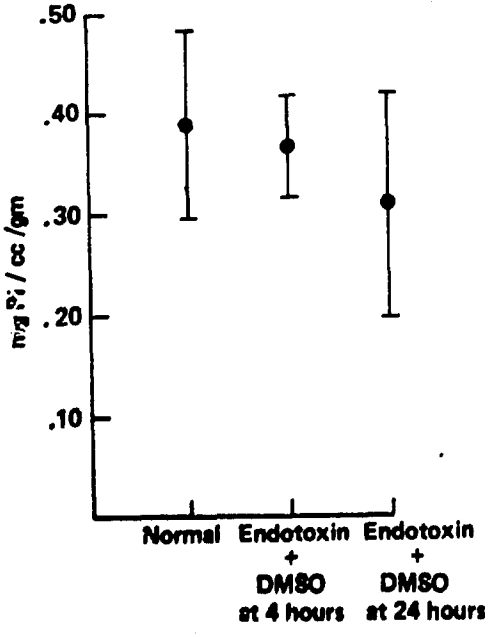

FIGURE 2. The mean P_i level was .390 ± .095 for normal rats. The amount of P_i assayed at 4 and 24 h was .367 ± .057 and .315 ± .113 for the rats given an intravenous injection of endotoxin (20 mg/kg) and an intraperitoneal injection of 6 ml/kg of 70% DMSO.

Discussion

The results of this study indicate that DMSO has a significant effect in preserving PCEC luminal ATPase activity and fine structure and survival in endotoxin-treated rats. Although the exact mechanisms remain speculative, data from this study and others suggest that the effect may involve the inhibition of the release of lysosomal enzymes and the scavenging of O_2 metabolites, such as superoxide anion ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$), produced by endotoxin-stimulated phagocytes.[6] Most organisms have active enzymes for the catabolism of H_2O_2 and $\cdot O_2^-$ (precursors of $\cdot OH$). However, there are no direct enzymatic mechanisms for the clearance of the highly cytotoxic $\cdot OH$. Thus, the reaction of DMSO with $\cdot OH$ may be an explanation for the protective mechanism.

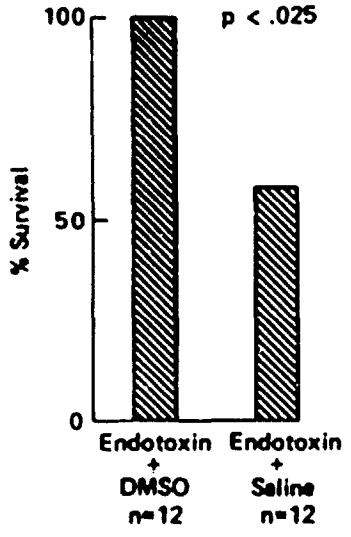

Figure 3. All 12 DMSO/endotoxin-treated rats survived 24 h (100%) compared to only 7 of the 12 rats (58%) treated with saline/endotoxin ($p < .025$).

References

1. Bisio, J. M., R. E. Breen, R. S. Connell *et al.* 1983. Pulmonary capillary endothelial dysfunction in hypoxia and endotoxemia. A biochemical and electron microscope study. J. Trauma. (In press.)
2. Coalson, J. J., L. B. Hinshaw & C. A. Guenter. 1970. The pulmonary ultrastructure in septic shock. Exp. Molec. Pathol. 12: 84-103.
3. Fiske, C. H. & Y. Subbarow. 1925. The colorimetric determination of phosphorous. J. Biol. Chem. 66: 375-400.
4. Harrison, L. H., L. B. Hinshaw, J. J. Coalson *et al.* 1971. Effects of E. coli sepsis in pulmonary hemodynamics and capillary permeability. J. Thorac. Cardiovasc. Surg. 61: 795-803.
5. Janoff, A., G. Weissmann, B. W. Zweifach *et al.* 1962. Pathogenesis of experimental shock. Studies on lysosomes in normal and tolerant animals subjected to lethal trauma and endotoxemia. J. Exp. Med. 116: 451-466.
6. Repine, J. E., J. W. Eaton, J. R. Anders *et al.* 1979. Generation of hydroxyl radical by enzymes, chemicals, and human phagocytes in vitro. J. Clin. Invest. 64: 1642-1651
7. Rose, H. J., R. R. Margolis & C. M. Jabs. 1972. Role of pulmonary microembolism in the hemodynamics of endotoxin shock. Surg. Gynec. Obstet. 135: 777-783.

8. SACKS, T., C. F. MOLDOW, P. R. CRADDOCK *et al.* 1978. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes: An in vitro model of immune vascular damage. J. Clin. Invest. **61**: 1161-1167.
9. WACHSTEIN, M. & E. MEISEL. 1957. Histochemistry of hepatic phosphatases at physiological pH with special reference to the demonstration of the bile canaliculi. Am. J. Clin. Pathol. **27**: 13-23.